Membrane Physiology

Jim Huettner

Objectives: This section is focused on two main topics: **(1)** The electrical properties of cells and **(2)** the role that energy balance plays in cellular processes, with emphasis on membrane transport. These two topics are fundamental to all of the subsequent sections of the course. Electrical signaling underlies the operation of the brain, the heart, and skeletal muscle. Membrane transport is required in all cells, but is particularly important for secretion in organs such as the kidney, lung, stomach, and pancreas.

The major goal of these sessions is to help you understand the basic chemical and physical principles that underlie electrical properties of cells and the movement of ions and other solutes across the cell membrane. By the end of the section you should understand the ionic basis of the resting potential. In addition, you should understand how energy balance will influence a cell's ability to move solutes across its membrane.

Teaching Goals – Lectures in this section will seek to explain:

- Why all cells need to express ion channels and the electrical consequences for the cell of expressing ion channels in its surface membrane.
- Why individual ions are not uniformly distributed across the cell membrane and how this unequal distribution is established and maintained.
- Why the unequal distribution of ions gives rise to a resting membrane potential. How the membrane's permeability to ions and the distribution of ions control the membrane potential.
- Why different mechanisms of membrane transport are required for different types of chemical compounds that the cell wishes to move across its plasma membrane and the physical properties that determine whether or not a particular compound will freely permeate the membrane.
- Why solutes that must bind to a proteinaceous carrier exhibit different kinetics of membrane transport than do freely permeable solutes.
- Why the energy available from ATP is not constant, but instead, depends on the concentration of reactants and products. How the energy of a solute concentration gradient is calculated.
- How the energy available from ATP and the energy required to transport solutes across the membrane compare. How the energy stored in an ion gradient can be used to drive the transport of another solute.
- The energetics and regulation of acid secretion in the stomach. Why interconversion of carbon dioxide and bicarbonate, coupled with chloride-bicarbonate exchange, is important for acid secretion and for the transport of products of respiration in the blood.
- How the membrane potential is measured experimentally and how the membrane

capacitance and resistance determine the time course of changes in membrane potential.

- The ionic basis of the action potential, the channel properties essential for generation of an action potential and the mechanisms that can terminate an action potential.
- Why there is a threshold for action potential generation and the factors that set the threshold.
- Why there is a refractory period following an action potential and why it determines the maximal action potential frequency.
- The gating states of voltage-gated sodium channels and the roles that each state plays in determining the action potential time course.
- Why the final effect of an action potential is to elevate cytoplasmic calcium.

Learning Objectives

Cell Membranes:

CM 1. Compare and contrast the mechanisms for solute transport across the plasma membrane

CM 2. Describe the energy requirements that drive the net "uphill" movement of solutes against a gradient in active transport

CM 3. Explain the factors affecting the direction of transport

CM 4. Describe the process of osmosis and the generation of osmotic pressure

Membrane Potential:

MP 1. Illustrate how membrane potentials are created

MP 2. Illustrate the roles of ion channels and pumps in maintaining the electrochemical gradients across the plasma membrane

MP 3. Explain the concept of diffusion potential, equilibrium potential, and electrochemical equilibrium

MP 4. Illustrate the driving forces and ionic conductance that affect ionic currents across a cell membrane

MP 5. Explain the resting membrane potential using the Goldman-Hodgkin-Katz equation

Action Potential:

AP 1. Explain how ion channels generate action potentials in excitable cells

AP 2. Illustrate the ionic basis of the different phases of action potentials

AP 3. Differentiate between absolute and relative refractory periods

AP 4. Describe the propagation of action potentials

AP 5. Examine the effects of hyperkalemia, hypercalcemia, and hypoxia on the resting membrane and action potential

Membrane Transport

Cells live in an environment of dilute salt water and contain within their cytoplasm a variety of dissolved molecules or **solutes**, including proteins and nucleic acids, but also many smaller charged metabolites like amino acids, Kreb's cycle intermediates, and ions. Cells need to retain essential metabolites inside the cell, but also must be able to move solutes, such as glucose, oxygen and ions, into or out of the cell across the surface membrane. Membranes are made of lipids that have a hydrophilic polar head group and nonpolar hyrophobic tails. The hydrophobic core of the membrane represents a significant diffusion barrier to many biological molecules and ions, but it is not a barrier to all compounds. Gases such as O_2 and CO_2 pass through the membrane by **simple diffusion** through the lipid bilayer, as do hydrophobic molecules such as steroid hormones and certain anesthetics, as well as many small polar molecules, including urea and ethanol. Water is able to cross biological membranes very rapidly so that cells are always at water equilibrium (the total concentration of dissolved particles is the same inside and out).

Larger polar compounds, such as sugars, are not freely permeable, nor are charged molecules, including amino acids and ions such as Na^+, K^+, Cl^-, etc. In order to cross the membrane, these impermeable molecules require a protein carrier or in the case of ions, an ion channel. **Carrier-mediated transport** mechanisms share several essential features that arise from the fact that the solute (**S**) directly binds to the carrier protein (**C**) in order to be transported: 1) The flux rate (***J***) is faster than expected from the lipid permeability of the molecule. 2) There is high selectivity for the molecule to be transported, but similarly shaped molecules may compete for the binding site. 3) The transport exhibits saturation kinetics.

Box 1 – Transport Kinetics

Extracellular solute (S_o) binds to a carrier facing the external environment (C_o) to form a solute-carrier complex (SC_o). The rate constants for solute binding (k_+) and unbinding (k_-) are typically much faster than the conformational change that delivers solute into the cell (S_i)

$$S_o + C_o \underset{k_-}{\overset{k_+}{\leftrightarrow}} SC_o \rightarrow S_i$$

At equilibrium the rate of change of SC_o (dSC_o/dt) equals zero:

$$(dSC_o/dt) = k_+ [S_o][C_o] - k_-[SC_o] = 0 \rightarrow k_+ [S_o][C_o] = k_-[SC_o]$$

$$\rightarrow (k_- / k_+) = ([S_o][C_o])/[SC_o] = K_m \rightarrow [SC_o] = ([S_o][C_o])/K_m$$

The fractional rate of flux will be the fraction of carriers that are complexed with solute:

$$J / J_{max} = [SC_o]/([C_o] + [SC_o]) \rightarrow J = J_{max} /(1 + [C_o]/[SC_o]) \rightarrow \mathbf{J = J_{max} /(1 + K_m/[S_o])}$$

This relation is very similar to Michaelis-Menten enzyme kinetics. Recall that:

- J_{in} is proportional to $[S_o]$ at low concentrations
- $J_{in} = J_{max} / 2$ when $[S_o] = K_m$
- $J_{in} = J_{max}$ at high solute concentrations

Carrier-mediated transport saturates at high solute concentrations

In some cases, the carrier protein simply allows the solute to pass across the membrane but does not supply energy to pump it against a concentration gradient. This type of passive flux would be termed Facilitated Diffusion. Proteins capable of active transport harness the energy in ATP or the energy stored in ionic gradients to move solutes up a concentration gradient.

Transport Energetics

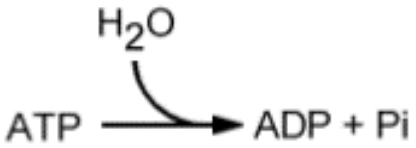
How does the energy available from hydrolysis of a molecule of ATP compare to the energy per sodium ion stored in the gradient of Na^+ across the membrane? For any reaction the change in free energy (**ΔG**) equals the energy in the products minus the energy in the reactants:

$\Delta G = G_{products} - G_{reactants}$ and **$G = R*T*\ln[C]$** where **R** is the gas constant (8.315 Joules / (°K * mole)), **T** is the temperature in °Kelvin (273 + °Celsius) and chemcal energy (**G**) is proportional to the natural logarithm of the chemical concentration.

For ATP hydrolysis:

$\Delta G = \Delta G° + 2.3*R*T*(\log([ADP]*[Pi]) - \log[ATP])$

Where $\Delta G°$ = -30 kiloJoules/mole is the free energy change under "Standard Conditions" of 1 Molar reactants and products at 20°C and pH 7.0



Surprising as it may seem, the energy available per molecule of ATP is not a constant value! Instead, it depends on the intracellular concentration of ATP, ADP and Pi. Specifically, the energy available depends on the ratio of products to reactants. All three concentrations might change over time or be different in different cell types. Therefore, the value we calculate will only be an estimate.

Different authors give slightly different values. Lodish et al. say that [ATP]in / [ADP]in ~ 10, but they do not give a value for [Pi]. Metzler (Biochemistry, Academic Press, NY 1977) says the ratio of ATP/(ADP * Pi), can be as high as 10^5. Alberts et al. quote ΔG values consistent with an ATP/(ADP * Pi) ratios of 600 to 20,000.

Box 2 – Energy per Molecule of ATP

We will use the following intracellular concentrations:

[ATP] = 4 mM, [ADP] ≈ 400 µM, and [Pi] ≈ 2 mM At 20°C: $2.3 * R * T$ = 5.6 kiloJoules / mole

So per mole of ATP hydrolyzed:

ΔG = - 30 kJ + 5.6 kJ * log ([2 X 10^{-3} * 4 X 10^{-4}] / [4 X 10^{-3}])

= - 30 kJ + 5.6 kJ * log (2 X 10^{-4})

= - 30 kJ - 21 kJ

= - 51 kJ per mole

Energy from a **single molecule** of ATP will use a different system of units. Remember that 1 Joule = 1 Volt * 1 Coulomb. Since a single electron bears a charge of 1.602 X 10^{-19} Coulombs, we define an "Electron-Volt" (eV) as the energy of one electron at a potential of 1 Volt and a milli-electron-Volt (meV) as the energy of one electron at a potential of 1 millivolt.

1 Joule = 1 V * (1000 mV / V) * 1 Coulomb / 1.602 X 10^{-19} Coulombs / electron

= 6.242 X 10^{21} meV Therefore the energy per molecule of ATP is given by:

(-51 X 10^3 Joules / mole * 6.242 X 10^{21} meV / Joule) / 6.02 X 10^{23} molecules / mole = **-530 meV**

The negative sign means that energy is released when ATP breaks down to ADP and Pi.

We conclude that in most cells each molecule of ATP can supply at least 500 meV of energy.

Now let's consider the energy per Na^+ ion for Na moving from outside to inside.

$\Delta G = G_{products} - G_{reactants} = G_{inside} - G_{outside}$

We must consider both the **electrical gradient** *and* the **chemical gradient**.

Box 3 – Energy in the Sodium Gradient

We will use the following concentrations and membrane potential (Vm)
Vm = -60 mV, $[Na^+]_{out}$ = 140 mM, and $[Na^+]_{in}$ = 14 mM (*Note:* these values will vary among different cells!) Na^+ ions are missing **one** electron and the constants 2.3 * R * T = 60 meV

Electrical Term:

ΔG = (# of electronic charges) * (mV_{inside} - $mV_{outside}$)
= +1e * (-60 mV – 0 mV)
= -60 meV The negative sign means energy is released moving out to in

60 meV is the energy required to move a charged ion (z=1) up a voltage gradient of 60 mV (assuming zero concentration gradient)

Chemical Term:

$\Delta G = 2.3 * R * T * (\log [Na^+]_{inside} - \log[Na^+]_{outside})$
= 60 meV * (-1)
= -60 meV The negative sign means energy is released moving out to in

60 meV is the energy required to move a molecule up a 10 fold concentration gradient (true for an uncharged molecule or for a charged molecule when there is no voltage gradient)

$\Delta G_{Total} = \Delta G_{electrical} + \Delta G_{chemical}$ = -120 meV per Na^+ ion

Summary

To pump a single Na^+ ion out of the cell up to a 10-fold concentration gradient and a 60 mV voltage gradient would require 120 milli-electron-Volts of energy. Hydrolysis of a single ATP molecule can provide at least 500 meV of energy - enough to pump four Na^+ ions.

A single Na^+ ion moving from outside of this cell to inside would be able to provide 120 meV of energy. That energy could be used to pump some other molecule, such as glucose, an amino acid, Ca^{2+} or H^+, up a concentration gradient.

Specific Examples

<u>#1 The Na+/K+ ATPase:</u> This protein carries 2 K^+ ions into the cell and 3 Na^+ ions out of the cell for each molecule of ATP hydrolized. If ATP provides 500 meV and 3 Na^+ ions require 3 * 120 meV = 360 meV, that leaves 140 meV for K^+. Will it be enough? K^+ moves from outside to inside. We will use the same resting potential, -60 mV, and the K^+ concentrations for mammalian nerve: $[K^+]_{out}$ = 5 mM, $[K^+]_{in}$ = 140 mM.

$\Delta G = G_{products} - G_{reactants}$ = $G_{inside} - G_{outside}$
$\Delta G_{electrical}$ = (+1e) * (-60 mV – 0mV) = -60 meV
$\Delta G_{chemical}$ = 60 meV * (log 140 – log 5) = +86.8 meV

$\Delta G_{Total} = \Delta G_{electrical} + \Delta G_{chemical}$ = -60 meV + 86.8 meV = +26.8 meV per K^+ ion

Why does K^+ require less energy than Na^+? Because K^+ is closer to electrochemical equilibrium when V_m = -60 mV than is Na^+. So, transport of 3 Na^+ out and 2 K^+ in requires a minimum of 360 meV + 53.6 meV ≈ 414 meV, leaving an excess of 500 meV - 414 meV = 86 meV provided by each ATP molecule. Some of that energy is probably used to drive the conformational changes in the transport protein; some of it will be given off as heat.

<u>#2 Na+ / Ca2+ Exchange:</u> This is an example of secondary active transport. The energy required to pump Ca^{2+} out of the cell comes from the Na^+ gradient. In Molecular Cell Biology, Lodish et

al. say that two (or possibly three) Na^+ are required per Ca^{2+}. Let's compare how low the internal $[Ca^{2+}]$ could be taken if two Na^+ were exchanged for one Ca^{2+} versus three Na^+ per Ca^{2+}. Ca^{2+} goes from inside to outside, V_m = -60 mV, $[Ca^{2+}]_{outside}$ = 1.5 mM and $[Ca^{2+}]_{inside}$ = ?

$\Delta G = G_{products} - G_{reactants} = G_{outside} - G_{inside}$

$\Delta G_{electrical} = (+2e) * (0\ mV - (-60\ mV)) = +120\ meV$

$\Delta G_{chemical} = 60\ meV * (\log 1.5 - \log ?)$

Energy available per Na^+ ion that enters is 120 meV and we assume all of that energy can be used to move Ca^{2+}.

2 $Na^+ \rightarrow$ 240 meV

240 = 120 + 60 * log (1.5/?)

120/60 = log (1.5/?)

10^2 = 1.5/?

? = 15 µM

3 $Na^+ \rightarrow$ 360 meV

360 = 120 + 60 * log (1.5/?)

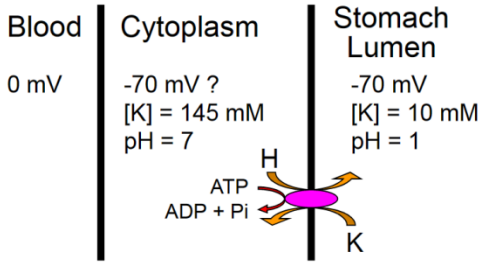
240/60 = log (1.5/?)

10^4 = 1.5/?

? = 0.15 µM

The internal Ca^{2+} concentration could be reduced 100-fold lower if three Na are exchanged per Ca^{2+} than if only two Na^+ are exchanged.

#3 Acid Secretion: Parietal cells secrete HCl into the stomach lumen. The cells use ATP to produce a million-fold proton gradient across their lumenal membrane. The potential difference across the resting epithelium, **from blood to lumen**, is known to be about 70 mV, with the stomach lumen more negative than the circulation. The resting potential of the parietal cells with respect to the circulation is not certain. For simplicity, we will assume that there is zero potential difference across the lumenal membrane. If the Class P ATPase brings in one K^+ with each cycle, how many protons can a single ATP pump into the stomach?



H^+ moves from inside to outside:

$\Delta G = G_{products} - G_{reactants} = G_{outside} - G_{inside}$

$\Delta G_{electrical} = (+1e) * (-70\ mV - (-70\ mV)) = 0\ meV$

$\Delta G_{chemical} = 60\ meV * (-1 - (-7)) = +360\ meV$ (remember pH = $-\log[H^+]$)

$\Delta G_{Total} = \Delta G_{electrical} + \Delta G_{chemical} = 0\ meV + 360\ meV =$ **+360 meV per H^+ ion**

K^+ moves from outside to inside:

$\Delta G = G_{products} - G_{reactants} = G_{inside} - G_{outside}$

$\Delta G_{electrical} = (+1e) * (-70\ mV - (-70\ mV)) = 0\ meV$

$\Delta G_{chemical} = 60\ meV * (\log 145 - \log 10) = +69.7\ meV$

$\Delta G_{Total} = \Delta G_{electrical} + \Delta G_{chemical} = 0\ meV + 69.7\ meV =$ **+69.7 meV per K^+ ion**

$\Delta G_{Total} = \Delta G_{proton} + \Delta G_{potassium} = 360\ meV + 69.7\ meV =$ **+429.7 meV total**

One molecule of ATP can supply about 500 meV. If about 430 meV is required to exchange one proton for one potassium, it is likely that only one proton is pumped per cycle.

Osmolarity and Tonicity Although cell membranes are hydrophobic, most cells remain constantly at water equilibrium, meaning that water will flow fairly rapidly into or out of the cell in order to equalize the internal and external solute concentrations. Many cells express proteins

called **Aquaporins** that form water-selective channels through the membrane and speed up this equilibration; but, even cells with few aquaporins equilibrate within a few seconds. If the external environment becomes more dilute water flows in and the cell will swell. If the external solution becomes more concentrated water will flow out and the cell may shrink. **Osmolarity** measures the total concentration of dissolved particles in a solution. **Tonicity** is the effective **Osmotic Pressure** (π) relative to blood plasma – it depends on total concentration as well as on the membrane permeability to each of the dissolved solutes.

Box 4 – Osmotic Pressure

The van't Hoff equation calculates the osmotic pressure from each solute in a complex solution:

$\pi = \mathbf{g} * \mathbf{C} * \sigma * \mathbf{R} * \mathbf{T}$ where **g** is the number of particles generated by each solute when it dissolves, **C** is the concentration, **R** is the gas constant, **T** is temperature in °K and σ is the reflection coefficient, which is an index of membrane impermeability to the solute. σ ranges from 0 for solutes that are very permeable to 1 for solutes that are very impermeable. An extracellular solution that is more dilute than the cytoplasm (**hypoosmotic**) is also **hypotonic** and will cause the cell to swell. An external solution that is more concentrated than cytoplasm (**hyperosmotic**) may also be **hypertonic**, but only if all the constituents are impermeable. Permeable solutes will pass into the cell and raise the cytoplasmic Osmolarity.

Membrane Potential

Cells tolerate a little bit of swelling or contraction, but not very much. Swelling in a hypotonic solution is particularly dangerous because the membrane may rupture. Since the environment can change quickly, early cells evolved ion channels as a way to make rapid adjustments to their internal osmolarity whenever external conditions change. The resting membrane potential is an unavoidable consequence of this strategy that cells use to handle changes in osmolarity.

Box 5 – Basic Electricity

Ions in solution are charged particles that move. **Charge** (Q) is measured in Coulombs. Movement of charge is **Current** (I), measured in Amps (A). **Voltage** (V) measures the energy difference for a charged particle in two different places, or the work required to move from one location to the other. **Ohm's Law** says that movement of charged particles through homogeneous material is proportional to the voltage difference from one end to the other. **Conductance** (G), measured in Siemens (S), is the proportionality factor between current and voltage. **Resistance** (R), measured in Ohms (Ω), is the inverse of conductance. Separation of (+) and (-) charges across an insulating barrier produces a potential difference or Voltage. **Capacitance** (C), measured in Farads (F), tells how much charge must be separated to give a particular voltage. Biological membranes have a specific capacitance of 1 $\mu F / cm^2$

Current (Amps) = Coulombs / sec Voltage (Volts) = Joules / Coulomb

Ohm's Law: I = G * V or V = I * R 1 Amp = 1 Volt / 1 Ohm

Capacitance (Farads) = Q / V = Coulombs / Volt

In addition to osmotic balance, cells face a second constraint that the internal and external solutions must be bulk neutral. Because opposite charges attract each other it takes energy to separate them. Therefore, the number of negative charges (anions) and the number of positive

charges (cations) must be equal on the millimolar scale. Cells at rest contain only a slight excess of negative charge that results in a negative membrane potential of -60 to -90 mV relative to the extracellular solution, which experimentally is "grounded" at 0 mV.
Ion channels are gated pores that allow the *passive* movement of ions across the membrane. The *direction* of flow is always toward **electrochemical equilibrium** which is defined by the **Nernst Equation**. In thermodynamic terms, ion channels essentially function as enzymes, lowering the energy barrier for ion transfer across the membrane. Ion channels cannot pump ions up an electrochemical gradient – only transport proteins with access to a source of energy, such as ATP hydrolysis, can pump against a gradient. Which ions are allowed through a particular channel (**selectivity**), as well as the rate of ion flow through an individual open channel (**unitary current**), are both determined by the pore properties of the channel protein.

Box 6 – The Nernst Equation
This equation defines the membrane potential at which a given ion will be in **electrochemical equilibrium**, meaning the total energy inside the cell is equal to the total energy outside.
Electrical energy = z * F * V where z is the valence, F is Faraday's Constant and V is Voltage.
Chemical energy = R * T * ln [Ion] where R is the gas constant, T is temperature in °K

$$z*F*V_{in} + RT*\ln [Ion]_{in} = z*F*V_{out} + RT*\ln [Ion]_{out}$$

$$z*F*(V_{in} - V_{out}) = RT*(\ln [Ion]_{out} - \ln[Ion]_{in})$$

$$V_{in}-V_{out} = E_{Ion} = (RT/zF) \ln ([Ion]_{out} / [Ion]_{in})$$

$$E_{Ion} = 60 \text{ mV} \log ([Ion]_{out} / [Ion]_{in}) \qquad \text{for } z = +1 \text{ at } \sim 30°C$$

Note that the Nernst Potential or Equilibrium Potential does not depend on how easy it is for the ion to cross the membrane, but only on the relative concentrations inside and out.

Ions flowing through open channels will eventually modify the cytoplasmic ion concentration; however, flux of charged ions also represents an electrical current that will alter the membrane potential. Only a small quantity of ions must cross the membrane to produce a significant change in membrane potential. Since charge (Q) = capacitance (C) * voltage (V), for a 1 cm^2 region of membrane to be charged to 60 mV would require

$1.0\ \mu F * 0.060\ V = 6 \times 10^{-8}$ Coulombs or 375×10^9 ions or 0.622 pico moles

Most cells are permeable to K^+ and Cl^- at rest because they express K-selective channels and Cl-selective channels that are open at rest. In contrast, cells at rest are relatively impermeable to Na^+ and to their charged internal metabolites, which have a net negative charge. As discussed in Box 7, the distribution of ions inside and outside a typical cell is such that K and Cl can be near electrochemical equilibrium at the resting membrane potential. Any ion not at equilibrium will flow toward equilibrium, if there is a conducting pathway to allow flux. So, for example, opening of a channel that is permeable to sodium will allow sodium to enter the cell down its electrochemical gradient. This entry of positive ions will depolarize the cell. As the membrane potential moves away from E_K and E_{Cl}, however, both K and Cl will begin to flow as well, with K flowing out and Cl flowing in toward their own electrochemical equilibrium point. Thus, the membrane potential is dynamic – it changes as a result of currents flowing through channels that open and close over time.

Box 7 - Ion Concentrations and Membrane Potential

Consider the distribution of ions typical for a cell at rest (Concentrations in mM):

	Out	In	E_{ion}
Na^+	146	15	+59.3 mV
K^+	4	120	-88.6 mV
Cl^-	150	5	-88.6 mV
Anions$^-$	0	145	
Total	300	300	

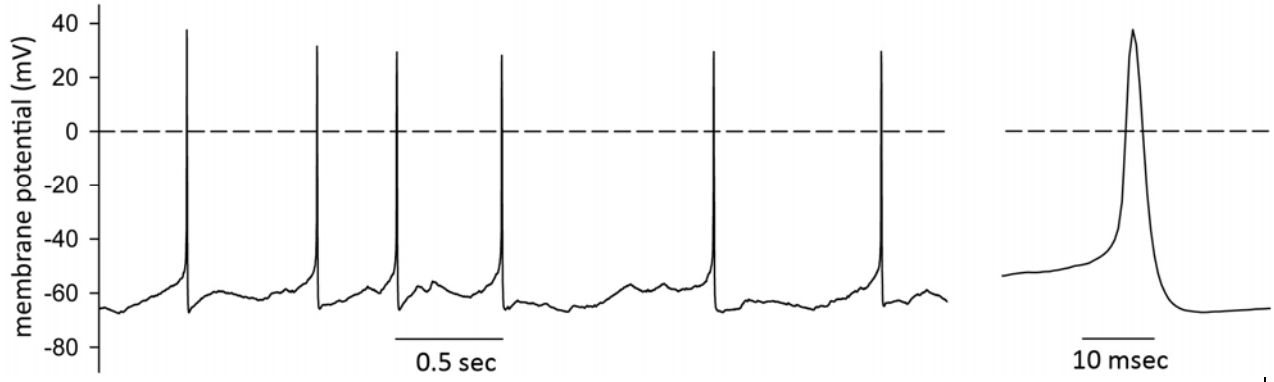
This distribution is in osmotic balance ($[total]_{out} = [total]_{in}$) and is neutral ([+] = [-] inside and out) at the bulk (mM) level. Nernst potentials for K^+ and for Cl^- are both approximately -89 mV, which would be the resting membrane potential of this cell if it were only permeable to K and Cl. Any cell that is only permeable to K and Cl will come to **Donnan Equilibrium**: flux of K and Cl will proceed following any perturbation until $[K]_{out} * [Cl]_{out} = [K]_{in} * [Cl]_{in}$ at which point $E_K = E_{Cl}$ Real cells have finite membrane permeability to Na^+ ions and the resting membrane potential is a "weighted sum" of Nernst potentials given by the **Goldman Hodgkin Katz Equation**:

$$V_m = 60 \text{ mV} \log \{(P_K[K]_{out} + P_{Na}[Na]_{out} + P_{Cl}[Cl]_{in}) / (P_K[K]_{in} + P_{Na}[Na]_{in} + P_{Cl}[Cl]_{out})\}$$

where P_K, P_{Na} and P_{Cl} are the relative permeabilities to K, Na and Cl, respectively. This equation describes a steady-state condition, *not* electrochemical equilibrium.

Action Potential

The most dramatic electrical signal is the **Action Potential**, a transient depolarization and repolarization of the membrane driven by voltage-gated Na-selective channels that open and then inactivate. Action potentials underlie heart beats, skeletal muscle contractions, and nerve cells signaling in the brain. Everything you do on a time scale of milliseconds to seconds is controlled and coordinated by action potentials. In virtually every case, the final effect of an action potential is to elevate cytoplasmic calcium, which then triggers secretion, muscle contraction, or other physiological responses.



Action potentials displayed at slow (left) and fast (right) time resolution

Recording Action potentials requires an electrical connection to the inside of the cell, which is achieved using a glass electrode, basically a hollow glass tube that has been heated and drawn down to a fine tip. The tube is filled with a salt solution that is compatible with the inside of the

cell. A silver wire connects the salt solution to a device for measuring voltage. The fine tip of the glass tube connects to the cell. In some cases, the glass electrode is sharp and is made to puncture the cell membrane. More recently, it has become popular to use a blunt glass electrode, seal it on to the cell membrane with suction and then rupture the patch of membrane encircled by the round electrode tip.

Once electrical contact with the cytoplasm is achieved, the membrane potential can be recorded passively or with appropriate equipment it is possible to clamp at a specified voltage and record current that flows through the membrane at that voltage, which is particularly useful for studying voltage-gated channels. With the blunt electrode "patch clamp" technique it is also possible to record the current that passes through individual open channels before the encircled patch of membrane is ruptured. This method allows for observation and measurement of conformational changes in an individual protein with time resolution of ~20 to 50 microseconds.

Passive Properties

Before talking about specialized channels that underlie action potentials we need to understand how the size and shape of a cell will influence the change of membrane potential over time. This analysis of Passive Properties will be true for *all cells* because the cell membrane acts as a capacitor and open ion channels in the membrane act as resistors, arranged "in parallel". A cell at "rest" has a steady membrane potential of about -70 mV. To change the membrane potential, current must flow across the membrane to deliver or remove charged ions. An excitable cell does this by opening ion channels; the ions change the membrane potential by flowing through the channels down their electrochemical gradients. During recordings we can change membrane potential by injecting current through a microelectrode.

Box 8 – A Simple Model Cell

The equivalent circuit for a spherical cell is a capacitor and resistor in parallel.

$I_{total} = I_R + I_C$ where $I_R = V_m / R$ and $I_C = \delta Q / \delta t = C * \delta V / \delta t$

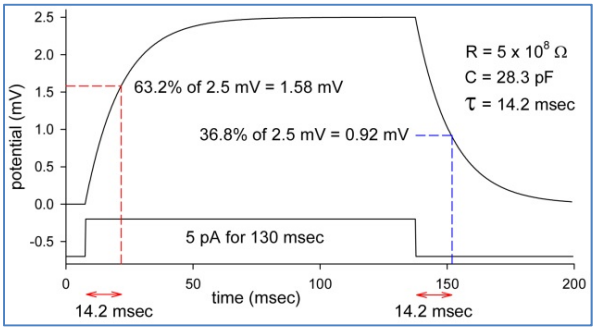
$I_{total} = V_m / R + C * \delta V / \delta t \rightarrow \delta V / \delta t = I_{total} / C - (V_m / R * C)$

$\rightarrow V_m = I_{total} * R * (1 - \exp(-t / \tau))$ where $\tau = R * C$ is the time constant

At equilibrium, when $t >> \tau$ then $Vm = I_{total} * R$ where $R = 1 / \Sigma G$ pores is the Input Resistance

At time $t = \tau$ the factor $(1 - \exp(-t / \tau))$ becomes $(1 - \exp(-1)) = 0.632$

Example: A 30 µm cell has 200 open channels each with 10 pS conductance. The surface area ($4*\pi*r^2$) is 2.83 x 10^{-5} cm^2 = 2.83 x 10^{-11} F. The input resistant is ($1/(200 * 10 \times 10^{-12})$) = 5 x 10^8 Ω. Time constant $\tau = R * C$ = 14.2 msec. Injection of current (5 pA) changes Vm to a new steady state $V_m = I * R$ = 2.5 mV. Suppose R changed as a result of ion channels opening or closing. How would that affect the change in membrane potential as a function of time? If R *increases* then $\tau = R * C$ will *increase* → Vm will approach its new value more *slowly* and the final change in Vm will be *greater* ($V = I * R$). If R *decreases* then $\tau = R * C$ will *decrease* → Vm will approach its new value *more quickly* and the final change in Vm will



be *smaller* (V = I * R). Suppose C were different (e.g., a larger or smaller cell but with the same R) how would that affect the change in membrane potential as a function of time? If C is *larger* then $\tau = R * C$ will be *larger* → Vm will approach its new value more *slowly* but the final change in Vm will be the *same* (V = I * R). If C is *smaller* then $\tau = R * C$ will be *smaller* → Vm will approach its new value *more quickly but* the final change in Vm will be the *same* (V = I * R).

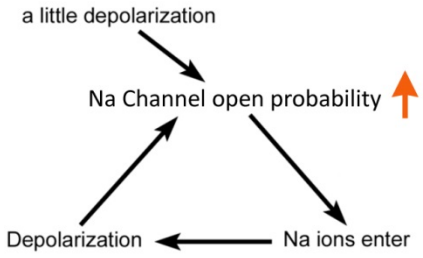
Excitable Cells

Cells capable of producing action potentials are said to be excitable. They express specialized channels with particular ionic selectivity and gating properties:

Ionic Selectivity - most channels show some preference among the various ions that are present in biological solution. Some channels are highly selective for Na^+, others are selectively permeable to K^+, others to Ca^{2+} and others to Cl^-. Some channels are permeable to cations (Na^+, K^+) but reject anions (Cl^-).

Gating - most channels are not open all of the time. In many cases, channel opening and closing (gating) is controlled by some external signal. Some channels are gated by binding a chemical; as a class, these are called **ligand-gated** channels and are responsible for synaptic transmission. **Voltage-gated** channels are controlled by the membrane potential and are essential for the action potential. The classic example is the voltage-gated Na^+ channel. This channel is closed at rest, is activated by depolarization, and spontaneously inactivates once it has opened.

Excitability is a consequence of inward current passing through voltage-gated channels activated by depolarization. Certain voltage-gated Na^+ and Ca^{2+} channels have the properties that are required to generate action potentials. These channels begin to open as the membrane potential depolarizes from rest. The resulting inward current further depolarizes the cell, which opens more channels and admits even more inward current. This regenerative cycle will continue until all channels are open. In this way, a small signal, which by itself only opens a few channels, will eventually lead to the opening of all Na^+ channels. Action potentials have a stereotyped shape because the regenerative depolarization cycle always leads to the same result - all Na^+ channels open. How can an action potential be terminated and the membrane potential returned to its resting value? Two mechanisms are involved:



Inactivation - The ability of some channels to enter a nonconducting state spontaneously after having opened. Na channels inactivate quickly, within a few milliseconds, whereas classical calcium channels inactivate slowly, if at all. Consequently, action potentials mediated only by Na^+ channels are terminated within a few milliseconds; action potentials involving calcium channels can be prolonged - this is a key distinction between action potentials in nerve and skeletal muscle (Na^+ channels) and those recorded in cardiac muscle (calcium channels).

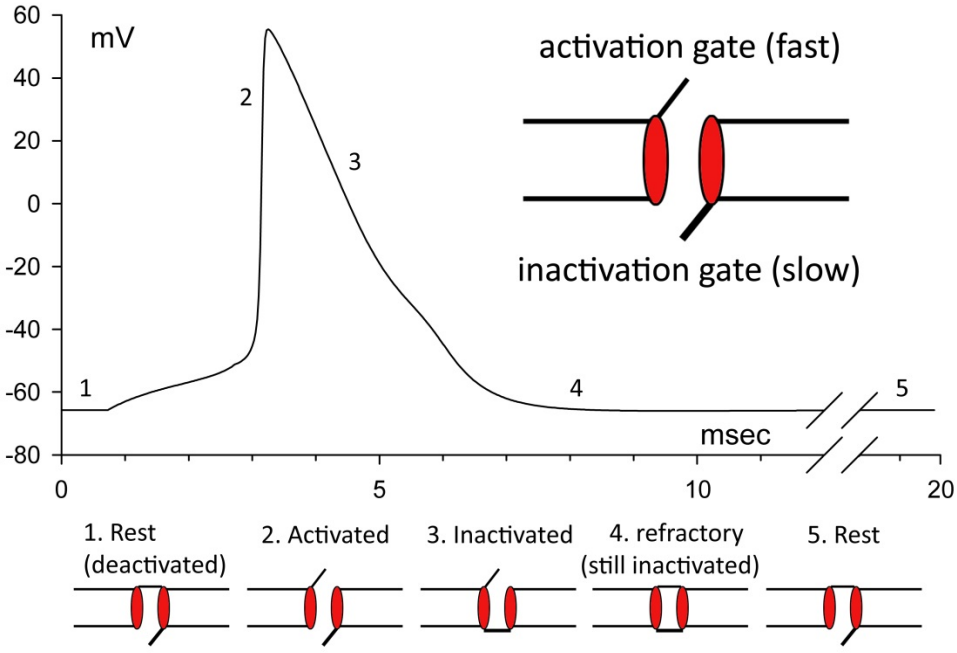
Voltage-gated K^+ channels - Activate slowly when the membrane potential is depolarized from rest. Outward current through these channels helps to repolarize the membrane in nerve and skeletal muscle.

Will every small depolarization from rest trigger an action potential? In most cells the answer is

no - there is a threshold level of depolarization that must be exceeded in order for an action potential to occur. This threshold depends on the relative rates of Na^+ entry and K^+ exit. An action potential will not be generated until enough Na^+ channels have opened such that Na^+ enters faster than K^+ leaves the cell. The threshold is not a fixed value. As channels open, close or inactivate the relative rates of Na^+ and K^+ flux will change. Remember that the rate of ion movement is measured as a current. For each ion the net current through the membrane can be described by a form of Ohm's law: $I = g * \Delta V$, where the conductance (g) depends on how many channels are open and ΔV is equal to $(V_m - E_{ion})$. There will only be net current if V_m is different from the ion's equilibrium potential. The quantity $(V_m - E_{ion})$ is often called the "driving force" on an ion to convey the fact that the current carried by the ion will increase as the membrane potential moves farther and farther away from that ion's equilibrium potential.

Gating States of the Na Channel

The most important feature responsible for fast action potentials is the ability of the Na channel to make transitions among three distinct states: closed, open, and inactivated. Ion permeation through the pore of the channel is prevented in both the closed and the inactivated states. The difference is that the closed state is available to be opened whereas the inactivated state will not open with depolarization. At rest, most Na^+ channels are in the closed state.

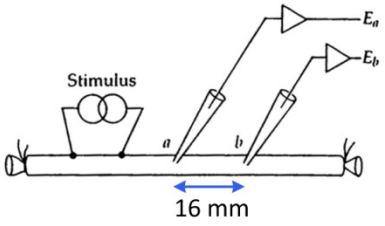


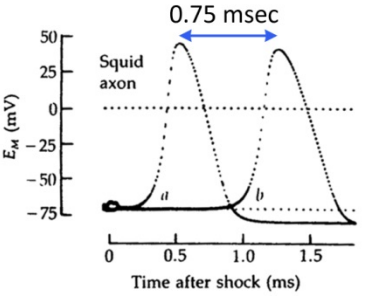
A small depolarization will cause channel open probability to increase. Once channels are open, depolarization also favors entry into the inactivated state, but this transition from open to inactivated is much slower than the closed to open transition. Channels can return from the inactivated to the closed state once the membrane repolarizes toward the resting potential. We can think of these transitions as two separate gates:

closed ⟷ open	"activation gate"	rapid
open ⟷ inactivated	"inactivation gate"	slow

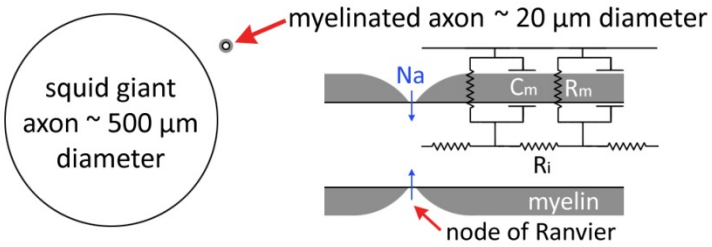
The cartoon depicts these gates as "trap doors" that block ions from gaining access to the channel pore. Depolarization causes the activation gate to open rapidly and the inactivation gate to shut slowly. In contrast, hyperpolarization (repolarization) causes the activation gate to shut rapidly and causes the inactivation gate to open slowly. When the action potential is over we see that both the activation and inactivation gates are shut. In this condition the channels cannot be reopened by a subsequent depolarization. Channels will slowly return to the resting state (activation gate shut and inactivation gate open) over a period of several milliseconds, as the inactivation gates open up. This refractory period, when channels are unavailable to pass current, will define the minimal allowed time between action potentials. In most cells it is possible to define an **absolute refractory period**, during which it is impossible to elicit an action potential, and a **relative refractory period**, when the threshold for action potentials is higher than in the resting state. The threshold for action potentials can also be influenced by changes in extracellular ion concentrations. **Hyperkalemia**, an increase in extracellular K^+, will depolarize cells and increase steady-state Na^+ channel inactivation, a process termed **Accommodation**. Changes in extracellular calcium modify excitability by shifting the voltage-dependence of Na^+ channel activation. Elevation of extracellular Ca^{2+} (**Hypercalcemia**) shifts the activation curve to the right, making threshold more positive and reducing excitability; whereas reduction in Ca^{2+} (**Hypocalcemia**) shifts the activation curve to the left, making threshold more negative and increasing excitability. Note that these changes in $[Ca^{2+}]_{out}$ will alter the equilibrium potential for Ca^{2+}, but these change in E_{Ca} are not responsible for the effects on Na^+ cannel gating.

Propagation



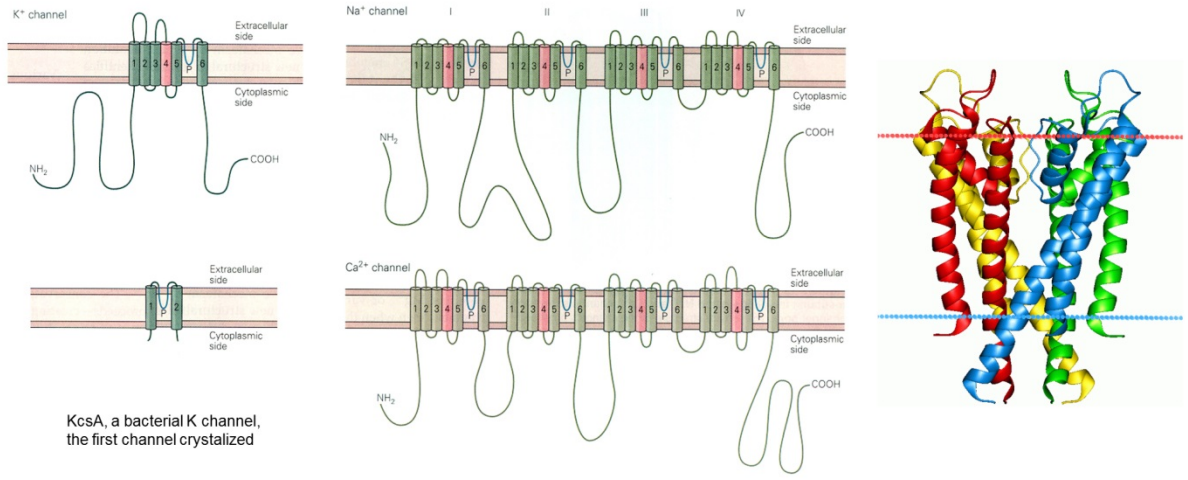


Action Potentials rapidly transmit information over long distances by propagating along nerve fibers as a traveling wave. **Conduction Velocity** depends on the axon diameter and whether or not the axon is wrapped by insulating layers of **myelin**. Large diameter unmyelinated squid giant axons conduct at about 20 meters/sec (16 mm / 0.75 msec = 21.3 m/sec), whereas smaller diameter myelinated mammalian axons can transmit at up to 120 m/sec. Increasing axon diameter lowers the internal resistance to current flow inside the axon (R_i), whereas myelin reduces capacitance (C_m) and increases resistance (R_m) to current flow out of the axon through the membrane. As for a spherical cell, the **membrane time constant** for an axon is given by $\tau = R_m * C_m$. For current injection at a point source the membrane potential will decay exponentially with distance determined by the **axon length constant** $\lambda = (R_m / R_i)^{0.5}$. Current travels farther and affects membrane potential at greater distance when the membrane resistance is larger and internal axial resistance is smaller. Na^+ ions enter at nodes of Ranvier where voltage-gated Na channels are concentrated.



Channel Structure

Many voltage- and ligand-gated channels have now been cloned and sequenced. Voltage-gated channel subunits share a number of topological features. The region labeled "P" in the diagram below contributes to the ion-conducting pore of the channel. Numbered cylinders are predicted regions of alpha helix that span the membrane. Potassium channels assemble as tetramers of homologous subunits, whereas sodium and calcium channels include four pseudo-subunits within single polypeptides. The fourth helix of each subunit, or pseudo-subunit, bears several positive charges that sense the membrane potential to control the activation gate.



Membrane topology of several pore-loop superfamily members KcsA ribbon diagram

In 1998 the crystal structure for a simpler bacterial potassium channel (KcsA) was solved by Rod MacKinnon and his colleagues (shown above). This structural information has greatly increased our understanding of ion permeation and has stimulated ongoing research on the molecular basis for channel operation. In addition, recent genetic/DNA analysis has allowed identification of numerous gain or loss of function mutations in ion channel genes (channelopathies), or in the genes encoding transporters or auxilary subunits. Together, these genetic abnomalities underlie many different human pathologies including forms of diabetes, epilepsy, cystic fibrosis, cardiovascular disease and numerous other conditions.

.

Study Questions: The Resting Membrane Potential

1. Name the different ways that molecules and ions might permeate cell membranes. What are the essential properties of a molecule that uses each mechanism?

2. Which mechanism(s) will be used by the following molecules?

a. Steroid hormones.

b. Glucose.

c. Anesthetics.

d. Positive ion.

e. Negative ion.

f. Vitamin E.

g. Water.

3. "Cells are always at water equilibrium!" What does this mean?

4. Define hypertonic, hypotonic and isotonic. Which of these three adjectives describes:

a. 100 mM NaCl.

b. 200 mM NaCl.

c. 150 mM NaCl.

d. 300 mM sucrose.

e. 100 mM Na_2SO_4.

5. What is the difference between the Nernst potential and membrane potential?

6. Under what conditions might the membrane potential equal a Nernst potential?

7. If the membrane potential is positive to the Nernst potential for a positive ion, in what direction will the ion flow through an ion channel in the membrane? What if the ion is negative?

8. Which ion is closest to equilibrium in a normal cell, K^+, Na^+, or Ca^{2+}?

Study Questions: Carriers and Pumps in Passive and Active Transport

1. What features distinguish carrier-mediated diffusion from simple diffusion?

2. What features distinguish passive carrier diffusion from active transport?

3. The Na^+ pump helps to keep $[Na^+]_{in}$ at 10 to 15 mM. Why doesn't the pump reduce $[Na^+]_{in}$ even lower?

4. What is a reasonable estimate of the energy in a molecule of ATP?

5. Can a molecule of ATP provide exactly the same amount of energy in every cell? Explain.

6. Could the energy available to a cell per molecule of ATP change over time? Why?

7. How much energy is required to pump an uncharged molecule up a 10-fold concentration gradient?

8. How much energy is required to pump a (+) charged ion up a 60 mV electrical gradient?

9. How much energy is required to pump a (+) charged ion up a 60 mV electrical gradient and up a 10-fold concentration gradient?

10. How much energy is required to pump a (+) charged ion **up** a 60 mV electrical gradient and **down** a 10-fold concentration gradient?

11. What is the situation described in the last question usually called?

12. If the stomach lumen and the parietal cell cytoplasm are both 70 mV more negative than the circulation, what is the potential difference across the lumenal membrane of the parietal cell?

13. In the lecture notes, we said $[K]_{cytoplasm}$ ~ 145 mM and $[K]_{lumen}$ ~ 10 mM, but we did not give values for Cl or Na. Given your answer to the last question, can you make any predictions about the concentrations of Na and Cl, and about the relative permeability of the lumenal membrane to these ions?

Study Questions: Ion Channels and Action Potentials

1. Describe and distinguish current, charge, voltage, resistance, capacitance. What are the units of each? What is a typical cellular amplitude for each?

2. Describe two means by which current passes through cell membranes.

3. "Inward current depolarizes the cell membrane." Explain this statement and the sign conventions behind it.

4. The direction of ion movement across a cell membrane depends on both the membrane voltage and the concentration gradient. How does Ohm's law for a membrane account for these two factors?

5. Why does an instantaneous input of current cause a slow change in membrane voltage?

6. Household wiring conducts electricity at nearly the speed of light (3×10^8 meters/sec), whereas the fastest nerve conducts at 100 meters/sec. Why are nerves so slow compared to metal wires?

7. Describe the gating states of voltage-gated sodium channels during an action potential. What feature of gating determines the refractory period?

8. Why are action potentials said to be "regenerative"?

9. What is the minimum requirement to generate an action potential? That is, how many different kinds of ion channels would be essential and what should their properties be?

10. Action potentials serve as triggers for diverse cellular outputs (secretion, muscle contraction, etc.). What is the common link between the action potential and these subsequent events?

Homework Problems and Answers from Previous Years

1. In lecture we said that digitalis affects the heart rate by inhibiting the Na^+-K^+ ATPase, which indirectly elevates $[Ca^{2+}]_{in}$. Suppose you have administered enough digitalis to double $[Na^+]_{in}$ from 14 mM to 28 mM ($[Na^+]_{out}$ = 140 mM). How much energy is available per Na^+ ion and how low could $[Ca^{2+}]_{in}$ be kept under these conditions if sodium / calcium exchange is solely responsible for Ca^{2+} pumping? Assume the following: **(1)** The cell is able to maintain V_m at -60 mV in spite of the change in $[Na^{2+}]_{in}$**. (2)** The exchanger uses 3 Na^+ per each Ca^{2+} ion.

$\Delta G_{electrical}$	=	- 60 meV
$\Delta G_{chemical}$	=	60 meV * (log 28 - log 140) = - 41.9 meV
ΔG_{Total}	=	- **101.9 meV**
3 Na^+	=	305.7 meV
305.7	=	120 + 60 * log (1.5 / ?)
185.7 / 60	=	log (1.5 / ?)
103.1	=	1.5 / ?
?	=	**1.2 µM**

Doubling of internal Na^+ from 14 mM to 28 mM caused nearly a 10-fold rise in the estimated internal Ca^{2+} concentration.

2. ATP is synthesized by mitochondria using the energy in the proton gradient across the inner mitochondrial membrane. Protons flow from the cytoplasmic side of the membrane (where pH = 7.0) into the matrix, which is the technical term for the compartment enclosed by the inner membrane (where pH = 7.3). If 3 protons must pass through the Class F ATPase per each molecule of ATP produced, estimate the voltage difference between the matrix and cytoplasm. (note - there is no voltage difference across the outer mitochondrial membrane). Assume that each ATP molecule requires 500 meV of energy.

H^+ moves from outside to inside.

ΔG	=	$G_{products} - G_{reactants} = G_{inside} - G_{outside}$
$\Delta G_{chemical}$	=	60 meV * (-7.3 - [-7]) remember pH = - log $[H^+]$
	=	60 meV * (- 0.3)
	=	-18.1 meV per proton or - **54.3 meV / 3 H^+**
$\Delta G_{electrical}$	=	$DG_{Total} - DG_{chemical}$
$\Delta G_{electrical}$	=	500 meV (needed per ATP) - 54.3 meV (available from DpH)
	=	445.7 meV (from 3 protons or 148.6 meV per proton)
148.6 meV	=	(+1e) * ($V_{in} - V_{out}$) * $V_{in} = V_{out} - 148.6$ mV

The matrix is at least 149 mV more negative than the cytoplasm (actually the voltage gradient has been measured at about 200 mV, matrix more negative than cytoplasm).

3. In class, we assumed that the resting potential of a parietal cell is -70 mV with respect to tissue fluid on the circulation side of the epithelium (cytoplasm is at same potential as stomach contents). **(a)** Calculate the energy required per pump cycle if the resting potential is actually -90 mV (cytoplasm is 20 mV more negative than stomach contents). **(b)** Repeat the calculation if mV is -50 mV (cytoplasm is 20 mV more positive than stomach contents).

a. H^+ moves from inside to outside.

$\Delta G = G_{products} - G_{reactants} = G_{outside} - G_{inside}$

$\Delta G_{electrical} = (+1e) * (-70\ mV) - (+1e) * (-90\ mV) = +20\ meV$

$\Delta G_{chemical} = +360\ meV$

$\Delta G_{Total} = \Delta G_{electrical} + \Delta G_{chemical} = 20\ meV + 360\ meV$

$= \mathbf{+380\ meV\ per\ proton}$

K^+ moves from outside to inside.

$\Delta G = G_{products} - G_{reactants} = G_{inside} - G_{outside}$

$\Delta G_{electrical} = (+1e) * (-90\ mV) - (+1e) * (-70\ mV) = -20\ meV$

$\Delta G_{chemical} = +69.7\ meV$

$\Delta G_{Total} = \Delta G_{electrical} + \Delta G_{chemical} = -20\ meV + 69.7\ meV$

$= \mathbf{+49.7\ meV\ per\ potassium}$

$\Delta G_{Total} = \Delta G_{proton} + \Delta G_{potassium}$

$= \mathbf{429.7\ meV\ required\ for\ 1\ proton\ and\ 1\ potassium}$

b. H^+ moves from inside to outside.

$\Delta G_{electrical} = (+1e) * (-70\ mV) - (+1e) * (-50\ mV) = -20\ meV$

$\Delta G_{chemical} = +360\ meV$

$\Delta G = -20\ meV + 360\ meV = \mathbf{+340\ meV\ per\ proton}$

K^+ moves from outside to inside.

$\Delta G_{electrical} = (+1e) * (-50\ mV) - (+1e) * (-70\ mV) = +20\ meV$

$\Delta G_{chemical} = +69.7\ meV$

$\Delta G_{Total} = 20\ meV + 69.7\ meV = \mathbf{+89.7\ meV\ per\ potassium}$

$\Delta G_{Total} = \Delta G_H + \Delta G_K = \mathbf{429.7\ meV\ required\ for\ 1\ H\ and\ 1\ K}$

***** Because this is an electroneutral exchange of one + charge for another, increasing or decreasing the potential across the lumenal membrane has an equal and opposite effect on H^+ and K^+. The total is the same at $V_{intracellular}$ = - 90 or - 70 or - 50 mV.